



# TRAP ASSAY

Version: 1

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Edited by: Eva Feldman, M.D.

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**Summary:** The use of total radical-trapping antioxidant parameter (TRAP) has recently been proposed to explore the antioxidant property of a plasma sample. This assay is a measure of oxidative stress in the animals. This protocol describes the procedure used by the AMDCC to measure TRAP.

## Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
100mM ABAP		Sigma	44,091-4
30mM PB pH 7.0			
Luminol		Amersham	RPN2106

## Protocol:

### Reagent Preparation:

**100mM ABAP\*:** Add 54.24mg to 2mL 30mM PB.

**Luminol:** Mix 2 reagent ½ & ½..

**IMPORTANT: Be sure light shield is in place**

### **Sample Preparation - DRG:**

- Remove 2 DRG from vial, cut in half and weigh. Do in duplicate.
- Add 30 $\mu$ L 30mM PB and sonicate on 4 on ice.
- Spin at maximum g's for 10 min @ 4<sup>0</sup>C.
- Remove sup and store on ice.

### **Performing the Assay:**

1. Using a **White Solid Bottom** plate, prepare plate by loading buffer for serial dilution. No PB in 1<sup>st</sup> well, 5 $\mu$ L PB in following 4 wells and 5 $\mu$ L in 3 control well. (Control is PB, Luminol and ABAP) (Dilutions, 1:1, 1:2, 1:4, 1:8, 1:16)
2. Prepare ABAP just prior to running assay by dissolving 54.2mg ABAP in 2mL PB. (Enough for 3 columns)
3. Load 5 $\mu$ L sample in wells 1 & 2. Mix the sample & PB in well 2 and remove 5 $\mu$ L and place in 3<sup>rd</sup> well with PB and so on. On last dilution discard 5 $\mu$ L.
4. Prepare Luminol in a 50mL conical tube and add 200 $\mu$ L per well.
5. Place plate in Fluoroskan and add 60 $\mu$ L ABAP per well and press **START**.
6. Read every 30 seconds for 20 minutes.
7. When reading is done, Select Process>Organize. Choose the appropriate data to organize (usually Measure1), then click **OK**. This rearranges the data into columns.
8. Save organized data as an Excel file into the TRAPAssay data folder. Use the naming convention trXXXXXX.xls, where XXXXXX is the date in yymmdd format.

\*ABAP = 2,2'-azobis(amidinopropane) dihydrochloride

\*TRAP = Total Radical-trapping Antioxidant Parameter

